

**CLAIMS:**

1. A method for non-invasively determining the expression of PKC isozymes in colonocytes of a patient comprising:  
5 directly isolating from a patient poly A+ RNA from feces, containing sloughed colonocytes; and  
assaying the isolated poly A+ RNA and determining the level, in the isolated A+ RNA, of mRNA encoding at least one PKC isozyme.
- 10 2. The method of claim 1, wherein the RNA is assayed using semi-quantitative RT-PCR.
3. The method of claim 1, wherein the RNA is assayed using biochip microarray technology.
- 15 4. The method of claim 1, wherein at least one PKC isozyme is selected from the group consisting of PKC  $\zeta$ , PKC  $\beta$ II, PKC  $\gamma$ , and PKC  $\beta$ I.
- 20 5. The method of claim 1, wherein the level of expression of PKC isozymes PKC  $\zeta$ , and PKC  $\beta$ II is determined.
6. The method of claim 5, wherein the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  is determined.
- 25 7. The method of claim 6, further comprising the step of comparing the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in other patients with known conditions.

8. The method of claim 7, wherein the level of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient is compared with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in at least two other patients, one with colon cancer and one without colon cancer.

5 9. The method of claim 6, wherein the level of PKC  $\zeta$  is determined using the primer pair having Sequence ID Numbers 7 and 8, and the level of PKC  $\beta$ II is determined using the primer pair selected from the group consisting of the primers having Sequence ID Numbers 11, and 12.

10 10. The method of claim 1, wherein the poly A+ RNA is isolated from rectal eluate obtained at the time of a colonoscopy.

11. A method for non-invasively detecting colonic biomarkers in a patient using fecal messenger RNA comprising:

15 directly isolating, from said patient, poly A+ RNA from feces containing sloughed colonocytes; and

assaying the isolated poly A+ RNA and determining the level, in the isolated poly A+ RNA, of mRNA encoding at least one colonic biomarker.

20 12. The method of claim 11, wherein the RNA is assayed using semi-quantitative RT-PCR.

13. The method of claim 11, wherein the RNA is assayed using biochip technology.

25 14. The method of claim 11, wherein said at least one colonic biomarker is a specific isozyme of PKC.

30 15. The method of claim 14, wherein the specific isozyme of PKC is selected from the group consisting of PKC  $\zeta$ , PKC  $\beta$ II, PKC  $\gamma$ , and PKC  $\beta$ I.

16. The method of claim 15, wherein the level of expression of PKC isozymes PKC  $\zeta$  and PKC  $\beta$ II is determined.

17. The method of claim 16, wherein the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  is determined.

18. The method of claim 17, further comprising the step of comparing the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in at least two other patients, one with colon cancer and one without colon cancer.

19. The method of claim 16, wherein the level of PKC  $\zeta$  is determined using at least one primer selected from the group consisting of the primers having Sequence ID Numbers 5, 6, 7, and 8, and the level of PKC  $\beta$ II is determined using at least one primer selected from the group consisting of the primers having Sequence ID Numbers 9, 10, 11, and 12.

20. The method of claim 11, wherein the poly A+ RNA is isolated from rectal eluate obtained at the time of a colonoscopy.

21. A method for non-invasively screening for colon cancer in a patient comprising:

detecting the expression of at least one specific biomarker in sloughed colonocytes in said patient's feces; and  
correlating the expression of said at least one specific biomarker with the presence or absence of colon cancer in said patient.

22. The method of claim 21, wherein said at least one specific biomarker is an isozyme selected from the group consisting of PKC  $\zeta$ , PKC  $\beta$ II, PKC  $\gamma$ , and PKC  $\beta$ I.

23. The method of claim 22, wherein the level of expression of PKC isozymes PKC  $\zeta$  and PKC  $\beta$ II is determined.

24. The method of claim 23, wherein the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  is determined.

25. The method of claim 21, further comprising the step of comparing the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in at least two other patients, one with colon cancer and one without colon cancer.

26. The method of claim 23, wherein the level of PKC  $\zeta$  is determined using at least one primer selected from the group consisting of the primers having Sequence ID Numbers 5, 6, 7, and 8, and the level of PKC  $\beta$ II is determined using at least one primer selected from the group consisting of the primers having Sequence ID Numbers 9, 10, 11, and 12.

27. A method for isolating poly A+ RNA from feces comprising the steps of:

homogenizing feces;

adding a dilution buffer to the feces to form a fecal homogenate;

centrifuging the fecal homogenate to create a supernatant;

adding oligo dT cellulose to the supernatant to form an oligo dT cellulose supernatant mixture;

mixing the oligo dT cellulose supernatant mixture;

centrifuging the oligo dT cellulose supernatant mixture to form a pellet;

resuspending the pelleted resin with a binding buffer;

centrifuging the resuspended resin to form a pellet and discarding the supernatant;

resuspending the pelleted resin with a wash buffer;

centrifuging the resuspended resin to form a pellet and discarding the supernatant;

resuspending the pelleted resin with an elution buffer;

eluting the poly A+ RNA by centrifugation;

5 precipitating the poly A+ RNA by adding a precipitation solution and chilling the resuspension; and

centrifuging the chilled resuspension to pellet poly A+ RNA.

28. The method of claim 27 wherein the amount of oligo dT cellulose  
10 added to the supernatant is in an amount equal to 10% of the starting fecal weight.

29. The method of claim 27 wherein the steps of centrifuging the oligo dT  
cellulose supernatant mixture to form a pellet, and resuspending the pelleted resin  
with a binding buffer are performed more than once.

15 30. The method of claim 27 wherein the steps of centrifuging the resuspended resin to form a pellet and discarding the supernatant, and resuspending the pelleted resin with a wash buffer are performed more than once.

20 31. The method of claim 27 wherein the elution buffer is about 65°C.

32. The method of claim 27 wherein the precipitation solution comprises  
about 60 µl 5M ammonium acetate, about 10 µg glycogen and about 2.5 vol 100%  
ethanol.

25 33. The method of claim 27 wherein the pelleted poly A+ RNA is further resuspended in a water/EDTA solution.

*Add 33*